Pedobiologia **33**, 355–360 (1989)
VEB Gustav Fischer Verlag Jena

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Mineralisation of ^{14}C-labelled plant material by *Porcellio scaber* (Crustacea, Isopoda)

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(Accepted: 89-04-18)

1. Introduction

The rate of decomposition of plant carbon in soil is enhanced by the presence of macrofaun such as earthworms and cranefly larvae (CHESHIRE & GRIFFITHS, 1989) and woodlice (REYES & TIEDJE, 1976a). Macrofauna have major effects on fungal and bacterial activities both directl through feeding and gut passage, and indirectly by affecting the microbial environment in litter an soil (ANDERSON, 1988). The enhanced carbon mineralisation in the presence of woodlice result from an interaction with soil microorganisms (REYES & TIEDJE, 1976a). Woodlice are unable t degrade cellulose and lignin themselves (BECK & FRIEBE, 1981; HARTENSTEIN, 1964; NEUHAUSE & HARTENSTEIN, 1978: NEUHAUSER *et al.*, 1978; REYES & TIEDJE, 1976a) but are thought t utilise cellulases produced by microorganisms in their gut (HASSAL & JENNINGS, 1975; HASSAL & RUSHTON, 1985) and can acquire cellulases from ingested microorganisms (KUKOR & MARTIN 1986). There is a characteristic bacterial flora in the gut, while faeces contain an increased bacterial fungal ratio compared with the ingested food (GRIFFITHS & WOOD, 1985; HANLON, 1981; INESO & ANDERSON, 1985; REYES & TIEDJE, 1976b; GUNNARSSON & TUNLID, 1986; WOOD & GRIFFITHS, 1988). Although the organisms in the faeces have an initially high respiration rat probably because of the soluble carbohydrates present (BIWER, 1961; MITCHELL, 1979; REYES & TIEDJE, 1976a), this declines rapidly and the overall decomposition rate of faeces is less than that the substrate (NICHOLSON *et al.*, 1966; REYES & TIEDJE, 1976a). The increased microbial activit of faeces may enhance the degradation of plant secondary defence chemicals, and allow mo nutrients to be absorbed upon reingestion of faeces (HASSAL & RUSHTON, 1985). The availability nitrogen, however, diminishes during coprography (GUNNARSSON & TUNLID, 1986).

Physical effects, either by moving leaf material to more favourable sites (HASSALL *et al.*, 198 or comminution (HANLON & ANDERSON, 1980), may be more important to overall decompositio than the chemical or microbiological changes in the faeces. Dispersal of microbial propagules another important function of soil macroinvertebrates (VISSER, 1985).

This present study has examined the interaction between *P. scaber* and soil microorganism during the decomposition of plant material. The mineralisation of C from uniformly ^{14}C-labell plant material, in the presence or absence of woodlice, or from faeces derived from that pla material was followed. The changes that occurred in the carbohydrate components of the substrat were also determined, in order to more fully understand the woodlouse – faeces – substra interaction.

2. Materials and methods

2.1. Invertebrates, soil and substrate

Porcellio scaber LATREILLE were collected from beech (*Fagus sylvatica* L.) woodland at the Macaulay Institu Soil of the Countesswells Association, Countesswells series (GLENTWORTH & MUIR, 1963) from permanent grassl was taken from a 0–15 depth and sieved ≦5 mm as required. Pondweed (*Lemna gibba* L.) was used to provid

uniformly ^{14}C-labelled substrate, and was grown aseptically in nutrient culture containing 0.1 % glucose or ^{14}C-glucose with a specific activity of 879 kBq g^{-1} (CHESHIRE, 1986) and freeze-dried prior to use. The specific activity of the dried plant material was 751.9 kBq g^{-1}.

2.2. Experimental design

To determine the effect of woodlice on soil microbial activity, moist soil, 75 g fresh mass (55 g dry mass), was placed in 24 7 cm × 9 cm × 10 cm PVC containers (Geneco Bio-Vessel, Bio-Genetech (Scientific) Ltd., Luton, England). Samples of 1 g of beech litter, collected from the L-layer in June, were added to the container and 12 containers were further amended with 5 *P. scaber*. All the containers were kept in a lighted incubator at 20 °C under a 12 h light: 12 h dark regime and watered to constant mass with distilled water. Three containers of each treatment (i.e. with or without *P. scaber*) were sampled after 1, 2 and 4 weeks. Total bacterial and actinomycete numbers were estimated by dilution plate counts using tryptone soya agar (Oxoid) and a streptomycete isolation medium (KUSTER & WILLIAMS, 1964). Numbers of ammonifying bacteria in peptone water (HARRIGAN & MCCANCE, 1966) and protozoa (DARBYSHIRE *et al.*, 1974) were estimated by most probable number techniques. Protozoan biomass was calculated by assuming an average volume (STOUT & HEAL, 1967) and a population density of 0.212 pg dry mass μm^{-3} (GRIFFITHS & RITZ, 1988).

Incubations with both unlabelled and ^{14}C-labelled. *L. gibba* were performed in 250 ml Erlenmeyer flasks fitted with gas tight lids with silicon rubber septa. In an experiment with unlabelled material, 10 g moist soil (6.97 g dry mass) was amended with either 150 mg *L. gibba*, 150 mg *L. gibba* plus 6 *P. scaber* that had been fed on *L. gibba* for 4 d, or was left as an unamended control. To obtain ^{14}C-labelled faeces, *P. scaber* and ^{14}C-labelled *L. gibba* were placed on a perforated metal plate in a gas tight desiccator. This allowed faeces to pass through the plate while woodlice and uneaten food were retained on it. The amount and radioactivity of CO_2 in the desiccator was measured every 7 d, as below, and at this time faeces were collected and freeze-dried and fresh ^{14}C-labelled *L. gibba* provided. Faeces were collected over a period of 2 months. The approximate digestibility of *L. gibba* was calculated as (I-F/I) × 100, where I = mass ingested, F = mass excreted. The digestibility of the chemical components of *L. gibba* was calculated as R − (E × P)/R, where R = amount in *L. gibba* (mg g^{-1}), E = amount in faeces (mg g^{-1}) and P = proportion of ingested *L. gibba* excreted. The specific activity of the faeces was 496.7 kBq g^{-1}. In an incubation with ^{14}C-labelled material, 10 g moist soil (7.17 g dry mass) was amended with 190 mg *L. gibba*, 190 mg *L. gibba* plus 6 *P. scaber* that had been used to provide faeces, or 100 mg faeces. Three replicates of each treatment were incubated at 20 °C (12 h light: 12 h dark). Gas samples were collected by syringe through the septum every day for the first 11 d, and then on days 13, 15, 18, 21, 25 and 28. The flasks being vented after each sampling. All the flasks were sampled after 28 d, the soils being analysed for carbohydrates and radioactivity and the gas samples for CO_2 and radioactivity as previously reported (GRIFFITHS & CHESHIRE, 1987). Briefly, individual sugars were assayed as alditol acetates by gas chromatography following acid-hydrolysis, and their specific activity determined by scintillation counting. The amount of CO_2 respired was determined by gas chromatography and its specific activity by dissolving 1 ml gas sample in 2 ml Carbosorb® and scintillation counting. Data were analysed by analysis of variance.

3. Results

3.1. Effect of woodlice on soil microbial activity

The total number of bacteria, and number of ammonifying bacteria and actinomycetes, in the soil underneath the beech litter were increased by the presence of *P. scaber* (table 1). The increases were transient in nature, with only the ammonifiers showing a trend of increasing numbers over the first 2 weeks when woodlice were present. Although the individual numbers of protozoa did not vary significantly, the total biomass of protozoa over the 4 weeks was greater ($\mathbf{P} < 0.001$) in the presence of *P. scaber* (table 1). In the incubation with unlabelled *L. gibba*, the total evolution of CO_2 over the 28 d increased from 2.65 ± 0.16 g C g^{-1} soil in the absence of woodlice to 3.80 ± 0.02 when *P. scaber* were present ($\mathbf{P} < 0.001$).

3.2. Digestibility of *L. gibba*

A total dry mass of 572.0 mg of faeces were obtained from 831.3 mg of ingested *L. gibba*. Thus, 31.2 % of ingested material was retained by *P. scaber*. The comparison between the content of ^{14}C-labelled sugars in food and faeces (table 2) indicated that the ability of *P. scaber* to digest plant sugars is in the order: glucose > arabinose > xylose > galactose.

Table 1. Mean numbers of total and ammonifying bacteria, actinomycetes and the mean biomass of protozoa in 3 replicates of soil incubated at 20°C either with or without *P. scaber*.

	Woodlice	Weeks				s.e. 12 d.f.
		0	1	2	4	
Bacteria	+	2.6	8.1[1])	1.8	1.9	1.1
$\times 10^{-6} g^{-1}$	−	2.6	1.7	1.9	1.2	
Ammonifiers	+	2.0	19.2	54.9[2])	2.4	6.4
$\times 10^{-3} g^{-1}$	−	2.0	1.5	1.4	3.3	
Actinomycetes	+	9.4	8.6[1])	8.0	7.2	1.1
$\times 10^{-5} g^{-1}$	−	9.4	4.2	4.8	7.9	
Protozoa	+	52.2	43.0[1])	34.3	42.8	7.6
$\mu g\ g^{-1}$	−		15.9	16.3	28.7	

[1]), [2]): Value significantly ($P \leqq 0.05$, 0.01 respectively) greater in the presence of *P. scaber*.

Table 2. The total activity (kBq g^{-1} l ± standard deviation) and percentage of the total activity of sugars in ^{14}C-labelled *L. gibba* and faeces derived from ^{14}C-labelled *L. gibba*, and the estimated digestibility of those sugars.

	L. gibba		Faeces		[%] digested[1])
	Activity	[%]	Activity	[%]	
Arabinose	11.7 ± 0.3	2.6	9.3 ± 1.6	4.5	45
Xylose	19.7 ± 1.8	4.3	24.3 ± 0.6	11.7	15
Mannose	9.6 ± 1.8	2.2	15.3 ± 1.0	7.3	+9
Galactose	13.3 ± 1.5	2.9	19.3 ± 0.2	9.3	0.3
Glucose	399.4 ± 16.5	88.0	140.4 ± 10.9	67.3	76

[1]) Assuming that 68.8% of ingested material is excreted, see text.

Table 3. Allocation of ^{14}C activity (kBq g^{-1}) in 3 soil samples amended with ^{14}C-labelled *L. gibba*, with or without *P. scaber*, or with ^{14}C-labelled faeces after incubation for 28 days.

	L. gibba		Faeces
	with *P. scaber*	without *P. scaber*	
Initial amount	19.925	19.925	6.982
Amount after 28 days in:			
Soil	6.108[a,b]	8.756[a]	3.998[a]
CO_2	2.170	1.620	0.200
Woodlice	2.367	−	−
	10.645	10.376	4.198
Unaccounted activity [%]	46.6	47.9	39.9

[a,b]: Value significantly ($P < 0.001$) less than initial amount (a) and than without *P. scaber* (b).

3.3. Mineralisation of ^{14}C

The presence of *P. scaber* increased the mineralisation of ^{14}C-labelled *L. gibba*, there being 13% less label remaining in soil when woodlice had been present (table 3). The difference could largely be accounted for by the activity in the bodies of the woodlice. There was a greater proportion of the initial ^{14}C-label remaining in soil containing faeces (57%) than *L. gibba* (44%). There was a

greater ($P < 0.05$) total amount of CO_2 evolved in the presence of woodlice (4.18 ± 0.46 g $C g^{-1}$) than in their absence (3.22 ± 0.11), while 1.17 ± 0.11 g $C g^{-1}$ soil was evolved from faeces. The proportion of the initial label lost as $^{14}CO_2$ was 2.9 % for faeces, 8.1 % for *L. gibba* and 10.9 % for *L. gibba* plus *P. scaber*.

3.4. Decomposition of ^{14}C-labelled sugars

^{14}C-labelled glucose was the only sugar to decompose at a significantly ($P < 0.05$) faster rate in the presence of *P. scaber* (table 4). It was galactose, however, that was lost more rapidly from faeces than from *L. gibba*, while glucose and arabinose present in faeces were degraded less rapidly than the same sugars present in *L. gibba* (table 4).

Table 4. Mean activity (Bq g^{-1} soil) of ^{14}C-labelled sugars remaining in 3 soil samples amended with ^{14}C-labelled *L. gibba*, with or without *P. scaber*, or with ^{14}C-labelled faeces after incubation for 28 days.

	Arabinose	Galactose	Glucose	Mannose	Xylose
+ *L. gibba* day 0	314.3	353.5	10,530	201.8	492.8
+ *L. gibba* day 28	144.0[b] (54 %)	240.5[c] (32 %)	1,399[a] (87 %)	276.2 (+37 %)	333.0[b] (32 %)
+ *L. gibba* + *P. scaber* day 28	116.0[b] (63 %)	235.7[b] (33 %)	814[a,1)] (92 %)	252.8 (+25 %)	305.5[b] (38 %)
+ faeces day 0	130.3	275.7	1,961	213.7	340.5
+ faeces day 28	76.8 (41 %)	106.0[b] (62 %)	500[a] (75 %)	137.8 (36 %)	220.7[c] (35 %)
s.e. 4 d.f.	18.7	17.9	107.3	21.5	20.8

[a,b,c]: Valuable significantly different from that on day 0 at $P \leqq 0.001$, 0.01, 0.05 respectively.
[1)]: Value significantly ($P \leqq 0.05$) lower with *P. scaber* present.
The percentage of the initial activity lost is given in parentheses.

4. Discussion

The increased numbers of bacteria and actinomycetes, the greater protozoan biomass and enhanced CO_2 production in the presence of *P. scaber*, agree with earlier observations that microbial abundance and activity are increased by woodlice. *O. asellus* similarly increased bacterial numbers in the substrate (ANDERSON & INESON, 1983: HANLON & ANDERSON, 1980), and also increased the respiration of sewage sludge (MITCHELL, 1979). Although *O. asellus* enhanced respiration initially, increased numbers reduced respiration while bacterial standing crop continued to increase (HANLON & ANDERSON, 1980). Differences in grazing pressure may explain the inconsistent stimulation of microbial numbers by *P. scaber* (table 1). The observed changes probably resulted from zones of intense microbial activity in the soil underlying the food/faecal material. Sampling would have taken unaffected soil as well as active zones, so diluting the apparent effect of the woodlice. The fact that statistically significant differences were still found indicates that the localised microbial activities must have been very considerable.

The digestibility of *L. gibba*, 31.2 %, was similar to the value of 31 % reported for leaf litter by *T. rathkei* [KUKOR & MARTIN, 1986], but somewhat larger than the 17 % reported for birch litter by *P. scaber* (HASSALL *et al.*, 1987).

Most of the sugars present in faeces were degraded less rapidly than the same sugars present in

L. gibba (table 4). There was also a greater proportion of labelled faeces remaining in the soil and a smaller proportion of $^{14}CO_2$ evolved from faeces than from *L. gibba* (table 3). This supports earlier observations that faeces decompose at a slower rate than the food material (NICHOLSON *et al.*, 1966; REYES & TIEDJE, 1976a). The different pattern of sugar decomposition of faeces from *L. gibba*, with or without woodlice, also indicated that faecal decomposition was not an important part of the enhanced decomposition of *L. gibba* in the presence of *P. scaber*.

The failure to detect significantly enhanced degradation of sugars other than glucose, in the presence of *P. scaber* (table 4), could have been because of their relatively low concentrations. Glucose accounted for 88% of all sugars present (table 2). The proportion of the initial label unaccounted for (i.e. the difference between ^{14}C in the soil, animal and CO_2 at the end of the experiment and ^{14}C added at the start), approximately 47% (table 3), was close to the value of 43% unaccounted for in a similar study (CHESHIRE & GRIFFITHS, 1989). This may have been the result of the formation of volatile hydrocarbons such as methane, but was unaffected by the presence of *P. scaber*. C mineralisation was also increased by *T. rathkei* (REYES & TIEDJE, 1976a) and *O. asellus* (HANLON & ANDERSON, 1980). The incorporation of 12% of the ^{14}C-*L. gibba* by *P. scaber* (table 3) was similar to the 13% of ^{14}C-cottonwood and 7% of ^{14}C-wheat retained by *T. rathkei* (REYES & TIEDJE, 1976a).

There appears to be a tendency for woodlice to increase C-mineralisation of recalicitrant substrates to a greater extent than more readily decomposable substrates. Thus, 24% more ^{14}C-labelled cottonwood was mineralised in the presence of woodlice (REYES & TIEDJE, 1976a), 13% more *L. gibba* in this study and 3% more yeast cells (REYES & TIEDJE, 1976a). This agrees with a similar observation for macrofauna in general (HEATH *et al.*, 1966). The enhanced mineralisation is probably not due to faecal decomposition, as indicated by this study. It seems reasonable to assume the action of woodlice in comminution, mixing of soil and substrate, enchanced microbial activity and digestive processes, all contributed towards a stimulation of C-mineralisation.

5. Acknowledgements

We thank Mr. H. SHEPHERD for radioactivity measurements and Mr. M. S. DAVIDSON and Miss K. A. WOOD for assistance.

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Synopsis: *Original scientific paper*

GRIFFITHS, B. S., S. WOOD & M. V. CHESHIRE, 1989. Mineralisation of ^{14}C-labelled plant material by *Porcellio scaber*. Pedobiologia **33**, 355–360.

Leaf litter was incubated in a mineral soil in the presence or absence of mature *Porcellio scaber*. The invertebrate caused an increase in the numbers of bacteria, ammonifying bacteria, actinomycetes and protozoa in the soil. The decomposition of ^{14}C-labelled *Lemna gibba* was significantly increased by the presence of *P. scaber* as determined by the total label remaining in the soil and the changes in sugars. ^{14}C-labelled faeces derived from *L. gibba* decomposed at a slower rate than the plant tissue from which it originated.

Key words: Woodlice, *Porcellio scaber*, decomposition, faeces, ^{14}C-*Lemna gibba*.

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